The Improper Use of Embedded Replicate Tags for Treatment Comparisons Among Samples of Coded-Wire Tagged Salmon

by

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Abstract.--An "embedded replicate" version of the standard coded-wire tag (CWT) has been used in management studies in recent years to provide assessment of variation in salmonid recovery data. These modified CWTs have sequence numbers which repeat through an entire lot of tags, thus producing subcodes within each CWT group. Embedded replicate CWTs were utilized in a study to evaluate relative survival of subyearling salmon passing through various structures at Bonneville Dam (Washington). Appropriate estimates of experimental error were obtained by releasing unique CWT groups over time (blocking), thus producing replicate experiments to detect the important potential sources of experimental variation. Treatment subgroups based on embedded replicate tags are not independent subsamples of treatment groups and can not be considered for experimental replication. Embedded replicate tags identify subgroups that exemplify isolative segregation of treatments. The use of such subgroups as discrete units for replication in statistical analysis of treatment differences violates the principle of interspersion. Inclusion of embedded replicate CWTs in fisheries research yields no useful information for comparison of treatment effects.

The coded-wire tag (CWT) (Bergman et al. 1968) has been used extensively on the Pacific Coast of the United States and Canada to provide uniquely marked groups of juvenile salmonids for research and management purposes (Johnson 1987). Since 1985, a refined version of the standard CWT, an "embedded replicate" CWT (Northwest Marine Technology Inc., unpublished) has been used to assess variation within salmonid recovery data. Modified CWTs have sequence numbers which repeat through an entire lot of tags, thus enabling assignment of subcodes within each group of test subjects.

Variability caused by uncontrolled conditions, secondary to the treatments being tested, affects the accuracy of assessments in fisheries science. We will use the general term "experimental error" to describe these chance components of variation (Winer 1962; Steel and Torrie 1980). Experimental error is the variation among "experimental units" which have been treated alike, where an experimental unit is the piece of experimental material to which one trial of a single treatment is applied (Petersen 1985). Experimental error includes a random error component, which affects precision, but may also have a systematic error component, or bias, affecting data accuracy. The random error component originates from lack of uniformity in the conditions or physical conduct of an experiment or the natural variability between experimental subjects (Steel and Torrie 1980). Natural variability between subjects is often termed "sampling error" and results from random grouping or "sampling" of subjects into discrete units (Steel and Torrie 1980; Petersen 1985). Experimental error is, therefore, at least no smaller than sampling error since it incorporates sampling error as well as other variance components. Often the magnitude of these error components is unknown.

Increased replication of treatments within an experiment functions to improve precision of hypothesis testing by reducing the effects of random variation (Hurlbert 1984). Estimates of experimental error can only be obtained through replication--by repeating the experiment it is possible to quantify experimental error unrelated to the planned comparisons and to develop confidence bands for the results (de Libero 1986). However, logistical constraints often make it difficult to conduct more than one test at the same time. When tests are conducted at different times (replication through time), experimental conditions and test fish physiology often change (Burnham et al. 1987). These additional sources of experimental error can be isolated from other experimental error components (i.e., sampling error and physical conduct of the experiment) by using a randomized block statistical design (Sokal and Rohlf 1981). Random administration of treatments to experimental units within replicate time blocks helps eliminate systematic bias. Restricted randomization guarantees proper interspersion of treatments over time (Hurlbert 1984).

In 1989, the National Marine Fisheries Service, in cooperation with the U.S. Army Corps of Engineers, conducted a study to evaluate relative survival of subyearling chinook salmon (*Oncorhynchus tshawytscha*) passing through various structures at Bonneville Dam (Washington) (Ledgerwood et al. 1990). Embedded replicate CWTs (Perry et al. 1990) were implanted in study fish in an attempt to obtain estimates of variance for each passage route on each release day. We will describe why juvenile recoveries of the embedded replicate CWTs did not provide any valid estimates of experimental error.

Methods

Study Design and Marked Fish Releases

More than 2.1 million juvenile chinook salmon were marked and released either via the Second Powerhouse turbines, Second Powerhouse bypass system, or spillway at Bonneville Dam during June and July 1989. Groups of 30,000 fish, each group marked with unique CWTs, were released at six differing sites (treatments) (Table 1) each of 12 days over a 30-day period.

Each of the 72 uniquely marked groups contained CWTs with embedded replicate subcodes 1, 2, and 3. These CWTs have binary codes identifying the release agency plus two identification numbers (Data 1 and Data 2) etched on the tagging wire, and, in addition to this standard format, a subcode (Northwest Marine Technology, unpublished). A total of seven subcodes were available in embedded replicate format; we chose to use three subcodes, allowing for three groups of 10,000 fish each. Expected recovery was 50 fish or more per subcode. The CWTs were implanted in fish with subcodes in sequential order and with the sequence of codes repeated. Marking fish with this type of tagging wire produced three embedded replicate CWT groups within each treatment group released each day (Table 1). Test fish were reared and marked at the Oregon Department of Fish and Wildlife's Bonneville Hatchery, Columbia River.

Test fish with the embedded replicate CWTs (the 72 independent groups) were held for 3 to 14 days, loaded onto trucks according to treatment assignment, and transported to the dam. After fish were acclimated to ambient river-water temperature, they were released as independent treatment groups on 12 different

days (blocks). After release at the appropriate location, fish were recovered using seines, 157 km downstream from the dam, near the Columbia River estuary at Jones Beach, Oregon (River Km 75).

Statistical Analysis

A randomized block analysis of variance was performed to compare differences among CWT recovery percentages for treatment groups. For this analysis, the three embedded replicate subcodes were nested within each independent CWT group and each release day was considered a block (Sokal and Rohlf 1981). The null hypothesis tested was that there were no differences in mean recovery percentages among treatments. Fisher's protected least significant difference (FPLSD) procedure was used to rank treatment means for significance (Petersen 1985); detectable differences were calculated by dividing the FPLSD by the average recovery percentage. Chi-square goodness of fit (single classification) was used to test the intrinsic null hypothesis that each subcode was recovered at an equal percentage (i.e., 33%).

Results and Discussion

Recovery Data

Study fish captured by seining at Jones Beach were sacrificed to obtain information from the CWTs; juvenile recoveries provided sufficient data for an evaluation of short-term survival differences between treatments. Over 18,000 tagged juveniles were recovered; total recovery averaged 0.86% of fish released. Among treatment groups released on the same day there was little evidence of significant difference in fish size, condition, timing, or riverine/estuarine distribution

at recovery (Ledgerwood et al. 1990). Tag data from returning adults will provide data for a final evaluation of survival differences.

Statistical Analysis

The analysis of juvenile recovery data used standard CWT information with the embedded replicates nested within each CWT group. For each block, a marked group of 30,000 fish received a single trial of one treatment. Therefore, variation between groups with the same treatment through time (adjusted for the block differences) constituted an estimate of experimental error. Variation between embedded replicate subcode groups within each CWT group reflected only natural variation between fish in those CWT groups (Schnute 1992), that is, an estimate of sampling error. The randomized block analysis of variance showed significant treatment differences ($F = 7.07$, $P < 0.01$, Table 2) at a detection level of 7.3% for $\alpha = 0.05$. Comparison of the mean squares for experimental error and sampling error revealed a significant amount of variation above sampling error ($F = 2.36$, $P < 0.01$, Table 2). Also, a substantial amount of variation over time was indicated by comparison of the relative sizes of block and experimental error mean squares in Table 2. Some sources of that variation were differences in fish size, age, and physiological condition, and differences in river flow, water temperature, predation, and recovery effort.

Embedded replicate subgroups did not fit the definition of experimental units because they did not individually receive a treatment application and therefore do not constitute repetition of the experiment (Schnute 1992). The variation in recovery percentages among subcodes was only a measure of sampling error. If the embedded

replicate subcode variation (i.e., sampling error mean square) were used erroneously to test for treatment differences, a 4.7% difference would have appeared significant. To increase the detection sensitivity of the study to 4.7% through increased replication would require the release of an additional 500,000 marked fish for each treatment--more than double the number of marked fish in the experiment (Table 3).

Expected recovery percentages for each embedded replicate code (33%) were significantly different from observed recovery percentages for only 4 of the 72 CWT groups. However, given a per-test error rate of $\alpha = 0.05$ for this analysis, we would expect 3.6 tests (72 x 0.05) to be spuriously significant. Since independent chi-squares and their degrees of freedom are additive, an overall chi-square was computed (overall chi-square = 135.25, df = 144, $P = 0.69$; Table 1); totals are presented below:

Embedded subcode:	1	2	3
Total recovered:	6,111	6,171	6,102
Percent of total:	33.2	33.6	33.2

It is not surprising that these recovery percentages are nearly identical because sequential implanting of codes would be expected to produce nearly perfect divisions of each treatment group. Procedures for holding, releasing, migrating to the sampling site, and recovering were also identical for within-treatment subgroups. Therefore, each subcode had equal probability to be affected by interactions with other variables inherent in the experiment.

Finally, the embedded replicate data provided no useful information concerning tag decoding error because standard binary codes are decoded differently than the replicate code. The embedded replicate CWTs were more complicated than standard CWTs and caused additional difficulty during the tag decoding process; 54% of the 884 initial tag decoding errors involved an error of the replicate code.

Conclusions

Potential sources of bias such as size-dependent mortality, migrational timing differences, or differences in spatial distribution of fish at the sampling site associated with treatment effects would not have been detected using embedded replicate tags to group fish as discrete experimental units. Nor would variations in procedures for holding and releasing treatment groups (which certainly did occur) have been detected by using embedded replicate tag data in this manner. Appropriate estimates of experimental error in this study were obtained by blocking over time in consideration of the need to identify important potential sources of experimental variation. Embedded replicate subgroups are not independent subsamples of the treatment groups and can not be considered replicates under the definition of independent subsamples of an experiment used by de Libero (1986). The embedded replicate subgroups do not represent experimental units (Steel and Torrie 1980); rather, they exemplify isolative segregation of treatments. Use of such subgroups as replicates in statistical analysis of treatment differences violates the principle of interspersion and constitutes "pseudoreplication" as defined by Hurlbert (1984). Improper use of embedded replicate CWTs in fisheries research complicates

the tag decoding process and yields no useful information for comparison of treatment effects.

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Table 1.--Estuarine recoveries of embedded replicate coded-wire tagged juvenile salmon from the Bonneville Dam Survival Study, 1989.

Release date	Subcode	Release site: Upper turbine	Lower turbine	Bypass	Front-roll	Spill-way	Down-stream	Daily totals
June 22	1	45	37	47	45	49	36	259
	2	45	46	65	60	66	30	312
	3	60	44	44	47	58	31	284
Chi-sq.[a]		3.00	1.06	4.96	2.62	2.51	0.64	14.78[b]
June 23	1	39	48	48	46	49	53	283
	2	68	32	34	49	57	54	294
	3	57	55	58	58	47	59	334
Chi-sq.		7.84	6.18	6.23	1.53	1.10	0.37	23.29
June 24	1	59	50	58	57	48	47	319
	2	58	62	39	51	52	43	305
	3	47	35	41	46	60	46	275
Chi-sq.		1.62	7.47	4.74	1.18	1.40	0.19	15.20
July 6	1	119	94	93	116	104	95	621
	2	106	108	92	121	104	120	651
	3	109	106	100	110	109	99	633
Chi-sq.		0.83	1.12	0.40	0.52	0.16	3.45	6.32
July 7	1	98	106	109	100	113	103	629
	2	108	116	112	107	124	127	694
	3	104	100	90	123	119	103	639
Chi-sq.		0.49	1.22	2.75	2.53	0.51	3.46	10.95
July 8	1	92	101	107	101	98	102	601
	2	92	95	109	81	122	109	608
	3	111	92	89	108	97	95	592
Chi-sq.		2.45	0.44	2.39	4.06	3.79	0.96	10.29
July 13	1	91	83	98	98	93	107	570
	2	80	87	76	98	89	98	528
	3	76	94	78	102	107	103	560
Chi-sq.		1.47	0.70	3.52	0.05	1.85	0.40	6.14
July 14	1	85	86	87	100	104	103	565
	2	76	91	80	97	87	93	524
	3	72	96	87	88	86	115	544
Chi-sq.		1.14	0.55	0.39	0.82	2.22	2.34	7.46

Table 1.--Continued.

Release date	Subcode	Upper turbine	Lower turbine	Bypass	Front-roll	Spill-way	Down-stream	Daily totals
July 15	1	86	92	75	98	99	97	547
	2	91	100	71	85	106	101	554
	3	72	94	55	85	99	102	507
Chi-sq.		2.34	0.36	3.34	1.26	0.32	0.14	7.77
July 20	1	97	91	88	84	117	121	598
	2	93	84	94	99	120	94	584
	3	116	80	105	100	115	118	634
Chi-sq.		2.97	0.73	1.55	1.70	0.11	3.95	11.00
July 21	1	85	83	92	86	102	114	562
	2	83	89	85	86	131	103	577
	3	91	94	79	82	96	116	558
Chi-sq.		0.40	0.68	0.99	0.13	6.39	0.88	9.48
July 22	1	84	96	90	77	116	94	557
	2	78	87	86	72	122	95	540
	3	77	89	75	88	109	104	542
Chi-sq.		0.40	0.49	1.44	1.70	0.73	0.62	5.34
Mean %[c]		0.83	0.83	0.80	0.86	0.96	0.91	0.86
Subtotals								
Subcode 1		980	967	992	1,008	1,092	1,072	6,111
Subcode 2		978	997	943	1,006	1,180	1,067	6,171
Subcode 3		992	979	901	1,037	1,102	1,091	6,102
Total number		2,950	2,943	2,836	3,051	3,374	3,230	18,384
Chi-square		24.90[d]	21.00	32.70	18.10	21.09	17.40	135.25[e]

[a] Chi-square = (observed - expected)^2÷expected, where: observed = observed catch of each of the embedded replicates for a given treatment group and release day, and expected = 33% of the total observed catch for all three replicates of the given treatment group and release day.

[b] The daily total chi-square is the sum of the individual treatment chi-squares and has 12 df.

[c] Mean percent recovery = total number recovered ÷ total number released x 100.

[d] The treatment total chi-square is the sum of the individual daily chi-squares for each treatment and has 24 df.

[e] The overall chi-square is the sum of all individual chi-squares and has 144 df.

Table 2.--Analysis of treatment effects for seine recoveries using a randomized block analysis of variance (ANOVA) design, where each day was considered a block, and statistical conclusions of homogeneity.

H_o: Mean recovery percentages for each treatment are equal.

ANOVA Table

Source	Sum of squares	df	Mean square	*F*	*P*
Block	9.8092	11	0.8918		
Treatment	0.6354	5	0.1271	7.07[a]	<0.01
Experimental error	0.9925	55	0.0180	2.36[b]	<0.01
Sampling error	1.0991	144	0.0076		
Total	12.5362	215			

Multiple Comparisons Method: 95% FPLSD[c] Intervals

Treatment	Count	Mean	Homogeneous groups[d]
Bypass	36	0.80	1
Lower turbine	36	0.83	1
Upper turbine	36	0.83	1
Frontroll	36	0.86	1,2
Downstream	36	0.91	2,3
Spillway	36	0.96	3

FPLSD = $t_{(\alpha = 0.05,\ df = 55)}[(2 \times MSE) / (b \times s)]^{1/2} = 0.0633$

where b = number of blocks (release days), s = number of embedded subcodes, and MSE = Mean Square for Error from Randomized Block ANOVA.

Detectable difference (%) = (FPLSD/Grand mean recovery percent) x 100 = 7.3.

[a] Calculated using the experimental error mean square.

[b] Calculated using the sampling error mean square.

[c] Fisher's protected least significant difference, calculated using the experimental error mean square.

[d] Treatment groups with identical numbers in this column signify that there were no significant differences in mean recovery percentages at $\alpha = 0.05$.

Table 3.--Release numbers needed to achieve a 4.7% detectable difference between treatment groups.

From Petersen (1985):

$$FPLSD = t_{(\alpha, df)} [(2\ MSE) \div (bs)]^{1/2}$$

where

$t_{(\alpha, df)}$ = the t coefficient corresponding to significance level α and df for MSE

MSE = Mean Square for Error from Randomized Block ANOVA

b = number of release days (blocks)

s = number of embedded replicate subcodes

Also, from the bottom of Table 2,

$$d = (FPLSD \div GM)\ 100$$

where

d = detectable difference

GM = grand mean recovery percent

Solving this equation for FPLSD,

$$FPLSD = (GM \times d) \div 100$$

Equations (1) and (2) together imply

$$GM \times d \div 100 = t_{(\alpha, df)} [(2\ MSE) \div (bs)]^{1/2}$$

Solving equation (3) for b,

$$b = (2\ t_{(\alpha, df)}^2\ MSE \times 100^2) \div (GM \times d^2 \times s)$$

where $\alpha = 0.05$; $t_{(0.05, 55)} = 2.004$; MSE = 0.0180; GM = 0.8643; d = 4.7; and s = 3

$$b = (2 \times 2.004^2 \times 0.0180 \times 100^2) \div (0.8643^2 \times 4.7^2 \times 3)$$

$$= 29.1$$

Therefore, approximately 29 release days (blocks) are needed to achieve a 4.7% detection level. Each treatment group released on a day consists of 30,000 fish, implying 870,000 fish per treatment would be required.

FPLSD = Fisher's protected least significant difference.